Algal Research 76 (2023) 103304

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

The effect of light intensity, spectrum, and photoperiod on the physiological performance of *Asparagopsis taxiformis* tetrasporophytes

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ARTICLE INFO

Keywords:
Asparagopsis
Methane mitigation
Aquaculture
Modeling
Photobioreactor technology

ABSTRACT

Over the last several years there has been increased interest in large scale cultivation of the red seaweed *Asparagopsis taxiformis* due to its ability to reduce methane emissions in ruminant animals. However, research focused on developing strategies to optimize culture conditions for this alga is still relatively scarce. Both light quality and quantity are widely known to differentially influence any alga's photosynthetic rates, metabolic budgets and ultimately biomass and bio-active compound production. Optimization of cultivation conditions for algae requires the understanding and fine-tuning of light parameters including Photon Flux Density (PFD), light spectrum, and photoperiod, as well as culture density which largely governs light quantity (PFD) and quality (spectrum) within the culture. Here we explore the effects of light intensity, spectral light distribution, and day length on the physiological performance of the tetrasporophyte stage of *A. taxiformis* in culture using a series of experiments. The results were used to characterize the optimal light and biomass density range for cultivation, and a model was developed to optimize the light path in a cultivation vessel. Spectral light optimization revealed a significant reduction in cyanobacterial contamination for cultures illuminated with blue LED light. Artificially extending light duration longer than 12 h per day did not improve algal growth. The insights gained from these experimental results may be used to inform the design of tank-based *A. taxiformis* cultivation systems to support high yield and stable production of this emerging commercial crop.

1. Introduction

1.1. Methane emissions mitigation and Asparagopsis as a possible solution

Methane is the second most abundant greenhouse gas after CO_2 but has a Global Warming Potential (GWP) 28 to 80 times greater than CO_2 calculated over 100 or 20 year time horizon, respectively [1,2]. However, while being such a potent greenhouse gas, the atmospheric lifespan of methane is relatively short (~12 years) in comparison to CO_2, which can be over hundreds to thousands of years. Considering its (1) abundance, (2) strong warming potential, and (3) short atmospheric lifespan, methane is emerging as a promising greenhouse gas to target for rapid climate change mitigation. This interest is evident in the global methane pledge signed by 105 countries at COP 26 in 2021, aiming for a 30 % reduction in methane emissions by 2030 compared to 2020 [3]. The livestock industry contributes 35 % of global anthropogenic methane emissions, primarily from enteric fermentation in ruminant animals [4]. Eliminating these emissions could reduce Radiative Forcing by approximately 0.22 W/m^2, equivalent to about 4.4 % of the projected Radiative Forcing at the end of the century [4].

Over the past decade, extensive research has focused on reducing methane emissions from enteric fermentation. Initially, 3-nitrooxypropanol (3-NOP) showed promise as a livestock supplement with a 30 % reduction in methane production [5,6]. However, in 2014, Machado et al. [7] explored various seaweed species as potential feed additives and found that *Asparagopsis taxiformis*, a red alga, exhibited the most potential for methane emissions reduction. Subsequent studies, including Kinley et al. (2016) [8], reported up to 98 % methane emission reduction in vitro and 80–98 % reduction in live cattle trials with 0.5 % or 0.2 % seaweed inclusion rates, without adverse effects on animal health or product quality [8–12]. Halogenated compounds, including bromoform, were identified as the bioactive compounds responsible for

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https://doi.org/10.1016/j.algal.2023.103304
Received 25 April 2023; Received in revised form 18 October 2023; Accepted 26 October 2023
Available online 30 October 2023

methane reduction [11]. Thapa et al. (2020) [13] recently elucidated the biochemical process for bromoform biosynthesis in *A. taxiformis*. Despite its effectiveness, widespread commercial availability of *A. taxiformis* for livestock remains a challenge. The bottleneck lies in developing scalable cultivation techniques to ensure stable year-round production [14]. The genus *Asparagopsis* has a tri-phasic hetermoprhic alternation of generations with a large macroscopic gametophyte stage and a small filamentous tetrasporophyte stage. While some groups have successfully cultivated the gametophyte stage in open ocean farms most other efforts have focused on land-based cultivation of the tetrasporophyte stage due to its ability to grow via vegetative propagation. While certain studies have demonstrated the potential for rapid growth rates of Asparagopsis tetrasporophytes when co-cultivated with fish in aquaculture systems [15–17], additional optimization remains imperative for the successful land-based cultivation of this seaweed.

Past studies have focused on the physiological responses of *Asparagopsis* to temperature [15,18,19] and nutrient supply [16,17,20,21] while the effect of light quality and quantity on growth and physiology has only been briefly discussed (by Mata et al. 2006 [22] and Figueroa et al. 2006 [23] for *Aspargopsis armata* and Zanolla et al. 2015 [24] for *A. armata and A. taxiformis*) from aquacultural and ecological perspectives, respectively. The flux of photosynthetically active photons available for photosynthesis has a major effect on a plant's energy and metabolic budgets, which are manifested in biomass production and growth rates. Optimization of cultivation for algae requires an understanding and tuning of light parameters including photon flux density of the photosynthetically active radiation (PAR), light spectrum, and day length (photoperiod), as well as culture density which governs to a large extent the light quantity (i.e. PAR) and quality (spectrum) within the culture (Masojídek et al. [25] and references within).

Photosynthetic organisms use photons of photosynthetically active radiation (PAR; 400 nm–700 nm) as an energy source for carbon fixation into organic matter. Generally, as PAR increases, photosynthesis and growth are elevated until a saturation point is reached (with maximal productivity P_{max}, at saturating irradiance I_k), from which any additional PAR does not translate into increases in photosynthesis, and when exposed to even higher PAR an inhibition of photosynthesis and growth occur (e.g., photoinhibition, etc.). This light-dependent response is commonly referred to as the P—I (Photosynthesis-Irradiance) curve. In dense algae cultures the incident photons are absorbed by the algae (and to a lesser extent also by water and other particles) as they penetrate into the cultivation vessel, thus the density of the algae in culture has a significant effect on the light availability (and resulting productivity) for the entire culture, with the highest biomass yield at the optimal cell density (OCD; g/l) [25]. For most realistic algae production systems, light attenuation results in drastic changes in light environments within a cultivation tank. In conventional outdoor tanks exposed to full sunlight, algae are exposed to varying and fluctuating light as they tumble through the tanks, traveling through four main light zones: (1) the surface layer, where the culture's surface is exposed to highest amount of light and photoinhibition may occur, (2) light saturation zone, where maximal photosynthetic rate is achieved, (3) the light limited zone, in which light is used with maximum efficiency, and (4) the dark zone in which irradiance is below the compensation level for photosynthesis and net photosynthesis cannot take place [26]. The light environment affecting the performance of an algal culture is therefore not solely determined by the irradiance at the surface of the cultivation unit, but rather by incident light intensity as well as light attenuation (determined by algae density and the biomass specific light attenuation coefficient K_a) and the length of the light path through the culture (dependent of tank dimensions and light direction).

Previous studies have explored the effects of light intensity and culture density on the temperate species *A. armata*. For this species, it was shown to reach P_{max} at $I_k \sim 150$ μmol quanta m^{-2} s^{-1} and started to show photoinhibition at ~200 μmol quanta m^{-2} s^{-1} when tested in the lab at densities of 0.8–1.6 gFW/l (reported as 0.2–0.4 gDW/l) [22]. However, when cultivated outdoors at much higher light levels and densities, photoinhibition was only recorded at biomass densities lower than 4 gFW/l (FW represent Fresh Weight), or for nitrogen depleted cultures, despite the very high PFD exposure exceeding 1500 μE at midday [22]. Figueroa et al. 2006 [23] reported higher I_k values of ~600 μE for dense (>4 gFW/l) *A. armata* cultures based on chlorophyll fluorescence measurements. Zanolla et al. 2015 [24] reported photosynthetic saturation at I_k between 70 and 170 μE for *A. taxiformis* and *A. armata* samples collected at various locations when tested for photosynthetic rate at their optimal temperatures. The OCD suggested by Schuenhoff et al. (2005) [15] for *A. armata* outdoor cultivation is 5 gFW/l. It is important to note that these rates and values may vary depending on acclimation of a given culture to a particular light environment.

Another important aspect of light availability is the spectral distribution of incident light. Photosynthetic machinery is set up to capture energy from photons capable of energizing pigment molecules mostly within the PAR range of 400–700 nm. However, different pigments definitionally have unique absorption spectra, and pigment abundance and composition will change with light quality. While chlorophyll *a*, the reaction center photosynthetic pigment, absorbs light mostly in the red and blue spectral ranges (absorption peaks distributed around 440 nm and 680 nm), other pigments obtained by specific algal taxa may absorb light within different spectral ranges [27]. Red algae, including *Asparagopsis*, are characterized by the abundance of the red pigment phycoerythrin, which absorbs light over a wide spectral range, roughly 460–570 nm (blue-green light), including spectral regions which are not available for any other photosynthetic pigments [28]. Based on this notion, we hypothesized that blue and/or green light with the right spectral range will assist *A. taxiformis* in outcompeting potential algal contaminants and will be effective in controlling contamination of non-red algae in *A. taxiformis* cultures.

The total amount of light energy available for photosynthesis is determined not only by illumination intensity but also by the illumination duration (i.e. day length). Extending the day length increases the Daily Light Integral (DLI) and may supply the algae with an abundance of light energy without causing exposure to excessive PFD. However, day length is also a common natural cue for reproduction and has been found to induce sporulation in *A. armata* sporophytes grown at low temperatures and short day-length (17 °C, 8 h day length, but may change with geographical origin [29]), leading to a potential complication in using day length manipulation for *Asparagopsis* cultivation unless reproduction is desired.

In addition to developing techniques to optimize productivity of *Asapragopsis*, there is also interest in determining how to optimize the production of the bioactive compounds responsible for reducing methane in ruminants [30]. Specifically, bromoform is the most abundant of the halogenated compounds that *Asparagopsis* produces and is believed to significantly impact methanogenesis in ruminant animals in a dose dependent manner. While other seaweeds produce bromoform and related compounds, the genus *Asparagopsis* has been shown to produce up to an order of magnitude more than other seaweed taxa. Because of this there is a need to better understand the factors that influence bromoform production, storage and release in seaweed. Interestingly, some studies have shown that exposure to high light can trigger the release of bromoform into seawater [31,32]. However, little is known about how optimizing culture conditions with regard to light quality and quantity may affect bromoform content in the tissue of the algae.

The goals of this study were to investigate the effect of light intensity, spectral distribution, and day length on the performance of *A. taxiformis* in laboratory experiments to inform optimization of light supply for commercial scale aquaculture. Specifically, we were interested in determining how the light environment (quality and quantity) affects the growth, photophysiology and bromoform concentration of this commercially important species to inform future aquaculture

development.

2. Materials and methods

2.1. Live material

A. taxiformis tetrasporophytes were collected from the field at various locations in San Diego County. These wild collected specimens were sorted, cleaned and isolated into uni-algal cultures (based on [33]) and held in a controlled environment at Scripps Institution of Oceanography (SIO), UC San Diego, La Jolla CA, USA. Cultures were grown in an aquarium room at ~21 °C, were constantly stirred by bubbling with air and were illuminated with 5 to 150 μmol photons m^{-2} s^{-1} PAR depending on their growth stage (lower for small individual branches and higher for dense larger volume cultures, detailed in table S1). Cultures were grown in closed vessels with natural seawater that was pumped from the end of the SIO pier, filtered to 0.2 μm using a series of cartridge filters and ultimately autoclaved to ensure sterile growing conditions. Seawater was amended with commercially available F/2 solutions (Proline F/2 Algae Food, weekly feeding of 65 μl/l from each fertilizer component, resembling half strength F/2) for major and minor nutrients. Cultures were monitored routinely for growth rates, and before each experiment, seed stocks were inspected microscopically to ensure culture cleanliness. Unless otherwise specified, clean and fast-growing cultures were chosen to be used in growth experiments.

2.2. Experimental setup

In order to get a better understanding of the effect of the light environment on the performance of *A. taxiformis*, three aspects of light conditions were investigated through growth experiments: (1) light intensity, (2) light spectrum, and (3) photoperiod. Growth experiments lasted one to five weeks in which the culture medium was replaced and culture parameters were measured weekly. In order to avoid the effect of increasing density across multiple weeks of experimentation, biomass density was brought back down to the initial stocking density (see below) weekly. High levels of nutrients, 200 μl/l F/2 (1330 μM NO_3, 56 μM PO_4 final concentrations), were used in experiments to ensure that nutrients were not limiting and to avoid increased high-light sensitivity for nitrogen starved cultures (as reported for red algae by [34]).

For each growth experiment, twenty four 500 ml Erlenmeyer flasks were inoculated with *A. taxiformis* biomass at 1 gFW/l density (unless otherwise specified, for the light-density experiments) and distributed on an water table illuminated with eight separated Hydra HD 64 (Aqua Illumination, USA) programmable light sources. Rigid opaque plastic sheets were used to separate bench spaces illuminated by each light source to allow independent control of eight individual light treatments (controlling PFD, light spectrum, and day length) each containing three culture flasks. Temperature was held at ~23 °C and monitored using HOBO temperature loggers (Onset, USA) deployed in 500 ml flasks next to the experimental flasks (one temperature-monitoring flask in each light chamber). Cultures were mixed and aerated by bubbling filtered (0.2 μm) air through 1 ml serological pipettes. Every seven days the biomass from each flask was drained, blotted dry using paper towels until no watermark seen (i.e. Fresh Weight; FW) and weighed to measure growth rate and return the biomass back to the original starting density. The harvested biomass was then used to quantify photosynthetic pigment concentration, bromoform concentration and contamination levels for each of the separate experiments-see below.

2.3. Light intensity experiments

Four light intensity experiments with eight light intensity treatments (PAR = 0, 28, 70, 150, 216, 380, 632, 1350, or 1550 μmol quanta m^{-2} s^{-1}) and four stocking densities (Density = 1, 2, 3, or 4 gFW/l) were conducted. Each experiment was started with *A. taxiformis* from three apparently clean cultures and consisted of twenty-four 500 ml flasks. Flasks were inoculated at a stocking density of 1, 2, 3, or 4 gFW/l and distributed in eight light chambers illuminated with Hydra HD 64 (Aqua Illumination, USA) light sources programmed for 10 K light color with PFD range 0–1550 μmol quanta m^{-2} s^{-1} (3 replicates for each light treatment in each experiment) on 12:12 light: dark cycles. Each experiment lasted for 14 days. Biomass from experimental flasks was weighed and sampled for bromoform, pigment concentration and contamination weekly (see details below). At the end of each week, biomass was brought down to the original density.

2.4. Spectral light experiments

Two experiments were conducted to examine the effect of light spectral distribution on contamination levels, as well as on growth rates and bromoform content. The first experiment was started with *A. taxiformis* from three apparently clean cultures and consisted of twenty-four 500 ml Erlenmeyer flasks. Based on the results of previous experiments, where the highest growth rates were achieved for *A. taxiformis* cultures inoculated at 1 gFW/l biomass density, and in order to minimize the effect of biomass pigmentation on in-culture light spectrum, a relatively low inoculation density was used for the following experiments. All flasks were inoculated at a stocking density of 1 g FW/l and distributed in eight light chambers illuminated by white, red, green, or blue LEDs with PAR intensity of 100 μmol quanta m^{-2} s^{-1} (6 replicates for each light treatment). LED light spectra were measured using JAZ spectral radiometer (Ocean Optics, USA) (Fig. 1). This experiment lasted for 35 days.

The setup for the second experiment was identical, but this time the cultures used for inoculation were cultures with known cyanobacterial contamination which was maintained at a consistent level before experimenting with the responses of the contaminants to changing light conditions. This experiment lasted for 28 days. Experimental flasks were weighed and sampled for bromoform, pigment concentration and contamination weekly. Biomass was then returned to the original density (1 gFW/l) and brought back to the experimental system. To get a better understanding of the different spectral light utilization by *Asparagopsis*, photosynthetic rates were measured as the change in oxygen concentration under the same LED light spectra used in the growth experiments.

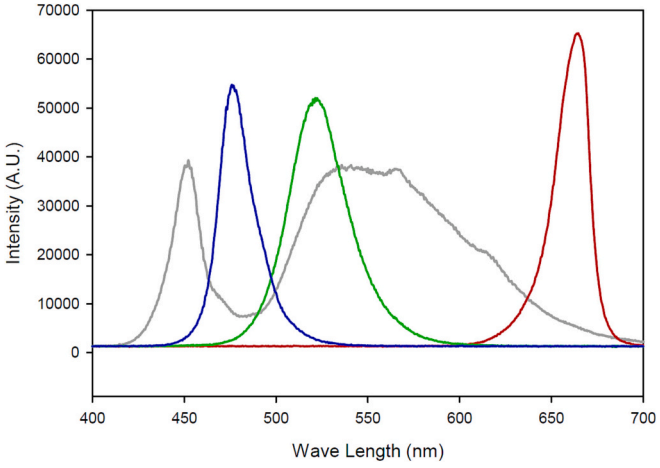

Fig. 1. Characterization of spectral light emission by the red, green, blue, and white LEDs used for the LED experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.5. Day length experiments

To determine the effect of day length on *A. taxiformis* growth and bromoform content, twenty-four 500 ml Erlenmeyer flasks were inoculated with three cultures at a stocking density of 1 g FW/l and distributed in eight light chambers illuminated by white LED with PAR intensity of 100 μmol quanta m^{-2} s^{-1} (6 replicates for each day length treatment). The selection of the inoculation density and PAR intensity outlined above was guided by the outcomes of prior light intensity experiments. In these experiments, it was determined that a PAR intensity of 100 μmol quanta m^{-2} s^{-1}, when applied to cultures at a biomass concentration of 1gFW/l, is approximately within the midpoint of the "light-limited region" on the photosynthetic-irradiance curve. This selection holds significance as altering light availability for cultures operating within the light-limited phase is expected to induce changes in performance (which might not be the case when dealing with cultures that are already light-saturated). The day length treatments consisted of 8:16, 12:12, 16:8, and 24:0 light:dark cycles. The experiment lasted for 28 days.

Biomass from the experimental flasks was weighed and sampled for bromoform, pigment concentration and contamination weekly, and measured for maximum photosynthetic quantum yield (Fv/Fm). Biomass was then brought down to the original density (1 gFW/l) and brought back to the experimental system. To assess reproductive state in the different day length treatments, eight 24 well plates were inoculated with *A. taxiformis* branches from the same three cultures that were used for the flask experiment (32 wells for each culture in each day length treatment) and placed under 90 % shade filter in the light chambers next to the flasks (resulted in 10 μmol quanta m^{-2} s^{-1} PAR). The well plates were inspected weekly (using a dissecting microscope) for the occurrence of sporulation and the percentage of sporulating wells was calculated for each well plate.

2.6. Response variables

2.6.1. Biomass measurements

To quantify growth rates of the algae, change in fresh weight (FW) was measured by sieving biomass through a 200 μm sieve and patting on a dry paper towel until no water marks were evident. FW was calibrated to Dry Weight (DW) by comparing FW to DW after 48 h drying at 65 °C yielding the relationship - DW = 0.35 FW.

Growth rate and biomass production were calculated following Eq. (1)

$$\mu\left(\%week^{-1}\right) = \frac{W_t - W_0}{W_0} \times 100, \text{and } Y\left(gFW\ liter^{-1}\ week^{-1}\right) = \frac{W_t - W_0}{V.} \tag{1}$$

Where μ is the weekly growth rate, Y is the biomass production, and W_0, W_t, V and t are the initial FW (g), final FW (g), culture volume (l), and culture time (in weeks), respectively.

2.6.2. Bromoform content

Tissue samples were taken to quantify bromoform content in seaweed across the different experiments. A total of 70–100 mg FW of biomass was sampled into 1.5 ml Eppendorf tubes and frozen at -80 C within 20 min of sampling. Frozen samples were sent on dry ice for analysis in the Agarwal lab at Georgia Institute of Technology. Algal biomass was lyophilized for 16 h. Freeze-dried biomass was soaked in MeOH for several hours before extraction with vigorous agitation on vortex mixer, centrifuged at 16,000 ×*g* for 30 min to remove debris, and an aliquot of supernatant was analyzed by GC–MS (1260G with 7890a MS; Agilent Technologies) in electron ionization (70 eV) mode using a DF-5 ms ultra inert GC column (30 m length, 0.25 mm width and 0.5 μM film thickness). Bromoform concentration was quantified based on calibration curves generated from a bromoform standard. The column temperature conditions were as follows: 40 °C for 3 min, increased to 200 °C at 10 °C/min and held for 1 min with a total run time of 20 min. Injection port, interface and ion source were kept at 250 °C, 300 °C and 230 °C, respectively. Helium was used as carrier gas at a flow rate of 0.9 ml/min.

In the spectral light experiments, we collected samples for bromoform analysis from each flask at the conclusion of experiment #2, resulting in a total of six bromoform samples per treatment. In contrast, within the light intensity experiment, we limited the bromoform analysis to a single sample per PAR level. This single-sample analysis occurred at inoculation densities of both 1 and 3 gFW/l during the first and second weeks of the experiment.

2.6.3. Photosynthetic pigment concentration

Approximately 100 mg FW biomass was collected from each experimental flask and frozen in a -20 °C freezer within 20 min from collection. Frozen biomass was subsequently freeze dried for 14–16 h. 5–10 mg DW of freeze-dried biomass was sampled into 1.5 ml centrifuge tubes where 0.1 ml of 0.5 mm glass beads were added and the mixture was homogenized using a bead beater at full speed for 1 min. Chlorophyll and carotenoid were individually extracted using *N*,*N*-dimethylformamide (DMF), while the phycobilins were extracted in separate tubes using phosphate buffer. For chlorophyll and carotenoid extraction, 1.5 ml DMF was added, and for phycobilins 1.5 ml phosphate buffer was added. Samples were incubated in the dark at 4°C for 24 h before reading. After 24 h, samples were centrifuged at 7000 rpm for 10 min at 10°C and 1 ml supernatant was measured in a UV-VIS spectrophotometer for spectral absorption. Chlorophyll and carotenoid absorbance levels at 480, 630, 647, 664, 680, 750 nm were measured, and pigment concentrations were calculated following eqs. (2–6) [35]:

$$Chl\ a\left(\frac{\mu g}{ml}\right) = (11.65A_{664} - 2.69A_{647}) \tag{2}$$

$$Chl\ b\left(\frac{\mu g}{ml}\right) = (20.81A_{647} - 4.53A_{664}) \tag{3}$$

$$Chl\ a\left(\frac{mg}{g}\right) = Chl\ a\left(\frac{mg}{ml}\right)*\left(\frac{1.5\ ml\ DMF}{gDW*1000}\right) \tag{4}$$

$$Chl\ b\left(\frac{mg}{g}\right) = Chl\ b\left(\frac{mg}{ml}\right)*\left(\frac{1.5\ ml\ DMF}{gDW*1000}\right) \tag{5}$$

$$Total\ carotenoids\left(\frac{mg}{g}\right) = \left(\frac{1000A_{480} - 0.89C_a - 52.02C_b}{245}\right)\left(\frac{1.5\ ml\ DMF}{gDW*1000}\right) \tag{6}$$

For phycobilins, absorbance levels at 455, 564, 592, 618, 645, and 750 nm was measured, and pigment concentrations were calculated following Eqs. (7)–(8) [36]:

$$PE\left(\frac{mg}{g}\right) = ([(A_{564} - A_{592}) - (A_{455} - A_{592})*0.2]*0.12)*\left(\frac{1.5\ ml\ buffer}{gDW}\right) \tag{7}$$

$$PC\left(\frac{mg}{g}\right) = ([(A_{618} - A_{645}) - (A_{592} - A_{645})*0.15]*0.15)*\left(\frac{1.5\ ml\ buffer}{gDW}\right) \tag{8}$$

2.6.4. Variable chlorophyll fluorescence

60–70 mg FW was sampled from each experimental flask and placed into a well in a 24 well plate containing sterilized and filtered seawater and incubated at room temperature in the dark for 1 h for dark acclimation. Photosynthetic quantum yield (Fv/Fm) was measured using a Diving PAM fluorometer (Walz, Germany) through the clear bottom of the well plate. Recognizing the potential for interference with the fluorescence signal due to the presence of the plastic plate, we implemented a procedure to minimize this effect during diving PAM measurements. Specifically, the instrument was zeroed on a well containing

only seawater (with no biomass) for each plate, thus reducing the impact of any potential interference. In order to assess the immediate effect of increasing light intensity on photosynthetic Electron Transport Rate (ETR), Rapid Light Curves (RLC) with light intensities of 0–770 μmol quanta m^{-2} s^{-1} were taken in the same manner using the Diving PAM fluorometer for samples of interest. The Rapid Light Curves were used together with growth and oxygen evolution data to evaluate the response of *A. taxiformis* to various light intensities (P—I curve).

2.6.5. Contamination assessment

Contamination in *A. taxiformis* cultures was assessed using an image analysis based contamination quantification [37]. Microscope slides were prepared with biomass from each flask. Microscope photographs were taken with Olympus SZX16 microscope at 2.5× magnification and bright field background using standardized capture settings. Contamination levels were quantified from microscope photos using an in-house developed image analysis protocol based on color threshold selections on ImageJ.

2.6.6. Photosynthesis/respiration

The photosynthetic and respiratory rates of *A. taxiformis* were measured across the experiments described above. Specifically, photosynthesis was measured across the light intensities (at 8 light intensities ranging 0–1200 μmol quanta m^{-2} s^{-1}) and light quality (white, red, green, and blue LEDs, Fig. 1) experiments. Photosynthetic rate was calculated by measuring the rate of O_2 production within a closed clear container, using a Hach Dissolved Oxygen Probe. Four oxygen electrodes were placed in 265 ml clear water-tight containers along with magnetic stir bars and 265 mg of *A. taxiformis* for a final density of 1 gFW/l. The containers were placed in a temperature-controlled environment with magnetic stirrers in the bottom. The temperature was held at 21.0 °C. The respiration rate was measured during an initial phase of 20 min in darkness. The photosynthetic rate was measured in 10-min intervals under different light intensities (0–1200 μmol quanta m^{-2} s^{-1}) or spectral distributions. Oxygen was measured every 30 s to quantify both respiration and photosynthesis. The photosynthetic rate was calculated as the change in O_2 per gram of *A. taxiformis* per minute.

2.6.7. Light attenuation measurements

In order to model the light environment in large scale vessels the PAR attenuation was measured for various *A. taxiformis* biomass densities. A 1000 ml glass beaker was filled with 0.2 μm filtered seawater to a height of 10 cm and illuminated from above by Amscope Fiber Optical Microscope Illuminator 150 W light source. Apogee MQ-510 PAR meter was used to record PAR values underneath the beaker. Light levels with no algae (seawater only) were used as PAR reference (100 % PAR). *A. taxiformis* biomass was added to the beaker in ~30 mg FW aliquots and continuously stirred by air bubbling in the beaker. PAR levels and the exact amount of biomass added were recorded after each biomass addition. To assess the light attenuation for different *A. taxiformis* morphologies this process was conducted using three types of biomass: (1) filamentous, (2) "puffball", and (3) frozen biomass that was finely diced to create a fairly homogenous culture when suspended in seawater.

At the end of each experiment the biomass was drained from the experimental beaker and dried at 65 °C for 48 h. The dry/fresh weight ratio was calculated for each experiment and used to convert fresh to dry weight. The light attenuation coefficient K_d (m^{-1}) was calculated as the difference in the logarithmic transformation of PAR levels divided by the light path (0.1 m).

$$K_d = \frac{ln(PAR_0) - ln(PAR_z)}{Z} \tag{9}$$

Biomass specific attenuation coefficients, K_a (m^2/gDW), were calculated by dividing K_d by biomass density X (in gDW/m^3).

$$K_a = \frac{K_d}{X} \tag{10}$$

2.6.8. Modeling and statistical analysis

The response variables for all experiments, including growth, contamination, Fv/Fm, and bromoform and pigment concentrations, were tested for significance using one way or two way ANOVA using SigmaPlot 13. Assumptions of normality and homogeneity were tested prior and all data satisfied assumptions.

Modeling the effect of light on weekly production of *Asparagopsis was* done as follows:

Weekly growth rates and photosynthetic performance (i.e. O_2 release and rETR) data were fitted by a modified hyperbolic model (in SigmaPlot 13) to generate a P—I curve for *Asparagopsis* .Then, in order to model the combined effect of density and light on growth rates in lab-based cultivation, growth rates versus density data for a given PAR were fitted with power series equations and the exponent, which was found to be related to PAR, was used together with the P—I curve to formulate the *ATAXlab* model predicting growth rates in laboratory scale cultivation for given density and PAR values.

Modeling of biomass production on larger scales as a function of biomass density and light path required the simulation of in-culture PAR for different depths and densities. In-culture PAR was calculated based on biomass specific attenuation coefficients, K_a (m^2/gDW), of 0.25 and 0.35 m^2/gDW (based on light attenuation measurements as described above), using Beer-Lambert model for light attenuation:

$$I_z = I_0\, e^{-K_a\, X\, Z} \tag{11}$$

Where I_z is PAR (μmol quanta m^{-2} s^{-1}) at depth *Z (m)*, I_0 is the incident PAR (μmol quanta m^{-2} s^{-1}), K_a is the biomass specific attenuation coefficient (m^2/gDW), and *X* is the biomass density (gDW/m^3). The P—I curve was then used to predict the growth rate for modeled PAR values in 1 cm increments and integrated over depth to predict the areal productivity of a cultivation tank for given depth (light path) and density.

3. Results

3.1. Light intensity experiments

Weekly growth rates and photosynthetic performance (i.e. O_2 release and rETR) were highly affected by PFD levels (Table 1) taking the shape of a classic P—I curve (Fig. 2a) fitted by a modified hyperbolic model (in SigmaPlot 13) (12).

$$\left(\mu\ (\%/week) = \left(\frac{117.4\ I_0}{79 + I_0}\right) - 0.062\ I_0 - 7.70 \right. \tag{12}$$

Where μ is weekly growth rate and I_0 is the PFD incident on culture's surface. Increased stocking density resulted in lower growth rates and higher saturation PFD but yielded the highest biomass production per volume when high PFD levels (>400 μmol quanta m^{-2} s^{-1}) were used (Fig. 2 c-d). Low density and high PFD levels also led to more frequent contamination outbreaks. Growth rate data for contaminated cultures are shown as cyan symbols in Fig. 3 but were discarded when fitting the data for modeling purposes (since these data do not represent *A. taxiformis* growth). Our limited bromoform data imply that increasing light intensity and high stocking density both resulted in lower bromoform content in *A. taxiformis* tissue (Fig. 2 e-f).

In principle, growth rates are expected to be affected by density due to within-culture self-shading that results in lower available light, and might therefore be calculated for different culture densities based on the P—I curve (measured using low density biomass) and biomass specific light attenuation. Nevertheless, modeling of the light environment within a lab flask is difficult due to the small size, complex light geometry, and the possible effect of fluctuating light. To overcome this

Table 1
Results of ANOVA tests for the Density-PAR, LED, and Day Length experiments. Only response variables where significant effect were detected are shown. PAR unit of μE is abbreviation for μmol quanta m^{-2} s^{-1}. Significant effects are shown in bold.

Response variable	Source of variation	DF	SS	MS	F	P	Post-hoc results
Light-density experiments results of Two Way ANOVA							
Growth rate (%/week)	Initial Density (gFW/l)	3	22,500	7500	35.491	**<0.001**	1 > 2, 3, and 4 gFW/l
	PAR (μE)	8	23,440	2930	13.865	**<0.001**	0 < 28–1550 μE 28 < 150–1550 μE
Biomass production (gFW/l/week)	Initial Density (gFW/l)	3	0.453	0.151	2.357	0.075	
	PAR (μE)	8	8.576	1.072	16.722	**<0.001**	0 < 28–1550 μE 28 < 150–1355 μE 70 < 632–1355 μE
LED Experiment #1 - Results of Two Way ANOVA							
Contamination (%)	LED spectrum	3	1257	419	9.871	**<0.001**	At the end of experiment: Red > Blue At day 21:Red > Blue, Green and White
	Time (days)	3	3077	1025	24.164	**<0.001**	Significant increase of contamination % with time for Red and White LEDs, no significant time effect on contamination % for other LEDs
	LED spectrum X Time	9	923	102	2.417	**0.014**	
LED Experiment #2 - Results of ANOVA (Two-way for LED and time and One-way for bromoform)							
Contamination (%)	LED spectrum	3	2967	989	21.410	**<0.001**	At the end of experiment: Red > Blue, Green and White Green > Blue
	Time (days)	3	1157	385	8.347	**<0.001**	Significant increase of contamination % with time for Red LED, no significant time effect on contamination % for other LEDs
	LED spectrum X Time	9	1145	127	2.754	**0.004**	
Bromoform content (% DW)	LED spectrum	3	1.847	0.616	3.069	0.051	
Day Length experiment							
Growth rate (%/week)	Time (days)	3	1353	451	2.199	0.096	
	Day Length (hrs)	3	5176	1725	8.408	**<0.001**	8 < 12, 16, and 24 h
	Day x Day Length (hrs)	9	1750	194	0.948	0.490	
Fv/fm	Time (days)	3	53,970	17,990	2.944	**0.039**	
	Day Length (hrs)	3	177,987	59,329	9.709	**<0.001**	24 < 8, 12, and 16 h
	Day x Day Length (hrs)	9	70,535	7837	1.283	0.263	

complexity, we based our predictions on the empirical light-density-growth data collected in the light-density experiments. In order to predict the combined effect of density and light on growth rates, growth rates versus density data for a given PFD were fitted with power series equations and the exponent was found to be related to PFD with Eq. (13) which was used together with Eq. (12) to formulate the *ATAXlab* model (Eq. 14) to predict growth rates in laboratory scale cultivation for given density and PFD value (Fig. 3 a-b).

$$exp = 3.55\ I_0^{-0.229} \tag{13}$$

$$\mu\ (\%/week) = \left(\left(\frac{117.4\ I_0}{79 + I_0} \right) - 0.062\ I_0 - 7.70 \right) X^{\left(-3.55\ I_0^{-0.229}\right)} \tag{14}$$

Where I_0 is the incident PAR (μmol quanta m^{-2} s^{-1}) and X is the biomass density (gFW/l).

Since PAR is rapidly attenuated due to absorption by algae while penetrating a cultivation tank, extrapolating growth-light-density predictions to cultivation scales larger than the laboratory flasks requires taking the length of light path through the culture into account. In order to allow for growth rate prediction in larger cultivation vessels, the *ATAXout* model was formulated based on a Beer-Lambert model for light attenuation through cultures. To calculate the light attenuation for any given density, light attenuation was measured in the lab for a wide range of densities and the biomass specific light attenuation coefficient was determined. K_a varied between 0.015 and 0.060 m^2/gDW with higher values for homogenous small filamentous morphologies in comparison to "puffball" growth forms (Fig. 2b and Supplementary Online Material). Eq. (12) was then used to predict the growth rate for the modeled PFD values in 1 cm increments and integrated over depth to predict the areal productivity of a cultivation tank for given depth (light path) and density. We present here the modeled result for indoor and outdoor systems, illuminated with 400 μmol quanta m^{-2} s^{-1} and 2000 μmol quanta m^{-2} s^{-1} respectively, both for light attenuation characteristic of filamentous cultures (K_a = 0.035 m^2/gDW) and of puffball cultures (K_a = 0.025 m^2/gDW) (Fig. 3 c-f). For full-sun outdoor conditions (PAR = 2000 μmol quanta m^{-2} s^{-1}) and low light attenuation (K_a = 0.025 m^2/gDW), our model results predict the highest productivity (328 gFW m^{-2} $week^{-1}$) at 1 gFW/l achieved with light path of ~90 cm. Slightly lower production rates (320–325 gFW m^{-2} $week^{-1}$) are predicted for densities of 3–5 gFW/l with a light path of 20–40 cm. The same system but with higher biomass specific light attenuation (K_a = 0.035 m^2/gDW) is predicted to produce significantly less biomass (up to 230 gFW m^{-2} $week^{-1}$ at densities of 1–5 gFW/l). Thinking of indoor cultivation systems illuminated at PFD = 400 μmol quanta m^{-2} s^{-1}, production rates of 250 and 180 gFW/m^2/Week can be achieved for cultures which absorb light at K_a of 0.025 and 0.035 m^2/gDW respectively. These maximal production rates are predicted to be achieved at densities of 5 gFW/l and light path of ~10 cm or at lower densities for deeper tanks (Fig. 3).

Light path length through a cultivation tank also influences the Light-Dark (L-D) cycles ratio and frequency. However, for cultivation tanks with a light path longer than a few cm the effect of L-D cycles on productivity is assumed to be negligible [38] and was not incorporated

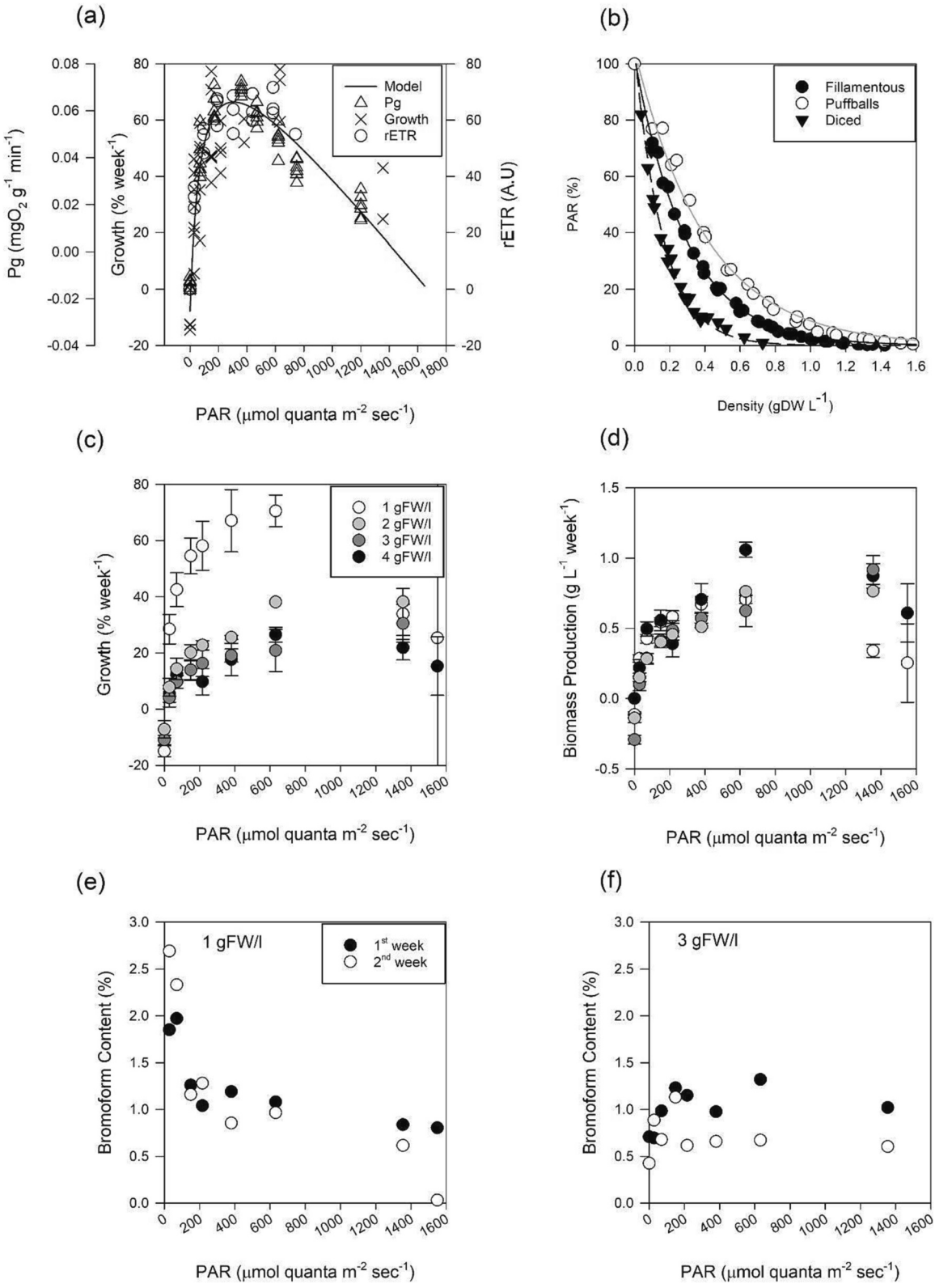

Fig. 2. The effect of light intensity (PAR) on *A. taxiformis* culture performance. (a) Photosynthesis-Irradiance curve. The blue line represents the modified hyperbolic model fitted to the experimental data. Triangles, crosses, and circles represent O_2 evolution, growth, and PAM data respectively. (b) PAR attenuation, measured as PAR% at 10 cm depth, for *A. taxiformis* cultures of different morphologies. (c-d) The effect of PAR and culture density on growth (c) and biomass production rates (d) of *A. taxiformis* cultures at lab scale (500 ml flasks). White, gray, dark gray, and black symbols represent 1, 2, 3, and 4 gFW/l biomass densities respectively. Error bars represent standard error. (e-f) Bromoform content of *A. taxiformis* measured after one week (black circles) and two weeks (open circles) under different PAR levels at two stocking densities (e, 1gFW/l, and f, 3gFW/l). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

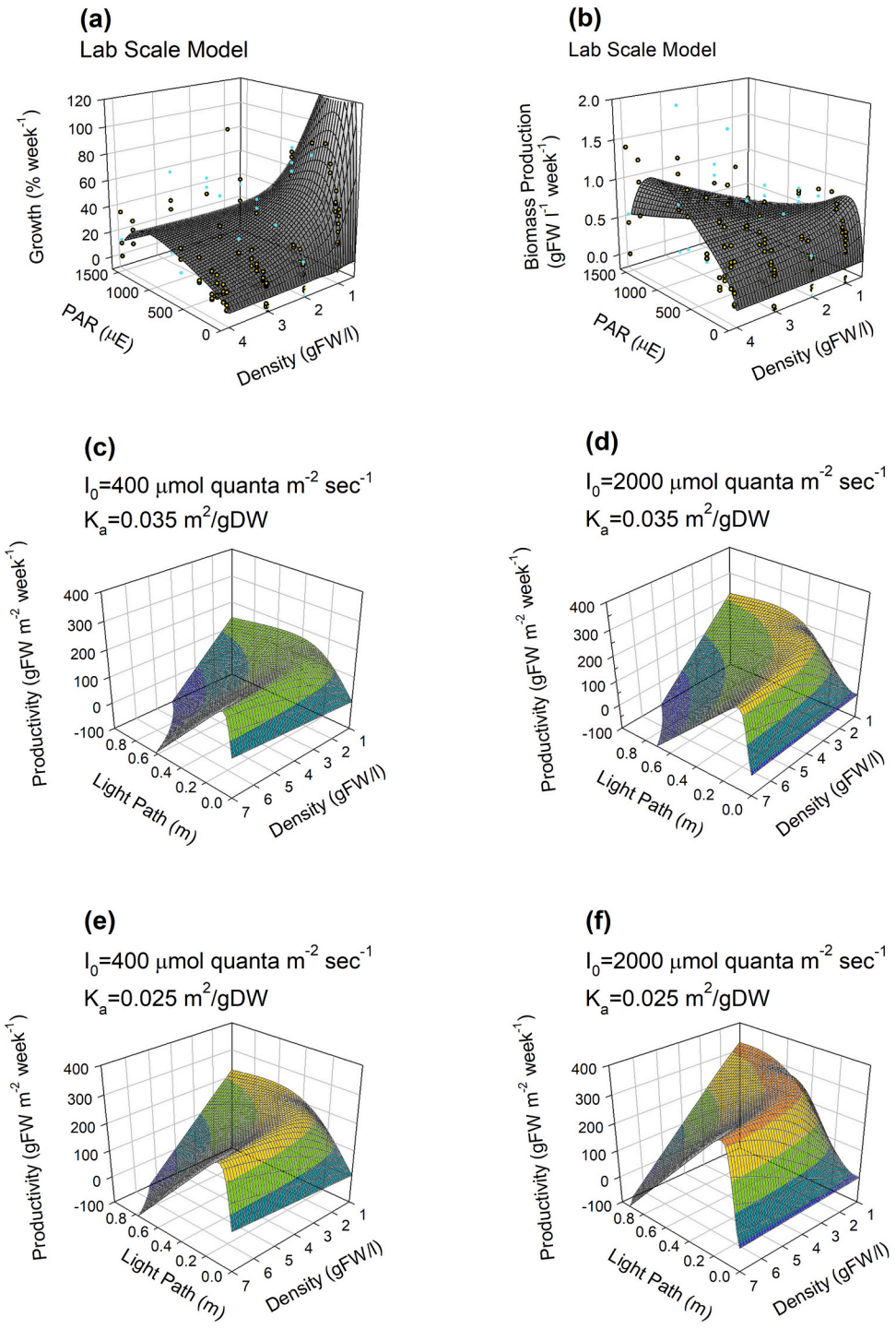

Fig. 3. ATAXlab and ATAXout model predictions. (a-b) ATAXlab growth rate (a) and biomass production (b) predictions (gray surfaces) for different PAR and density levels. PAR unit of μE is abbreviation for μmol quanta m^{-2} s^{-1}. Yellow and cyan symbols represent clean and contaminated cultures respectively. (c-f) *ATAXout* Model predictions for density and light path effect on productivity of a cultivation tank illuminated with 2000 μmol quanta m^{-2} s^{-1} (d, f) and 400 μmol quanta m^{-2} s^{-1} (c, e). Two biomass specific light attenuation coefficients (K_a) were used to represent normal (c, d; K_a = 0.35 m^2/g) and low (e, f; K_a = 0.25 m^2/g) light absorbing biomass. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

into the model.

3.2. Spectral light experiments

Two experiments were conducted to assess the effect of light spectrum on growth, contamination susceptibility, pigment content and bromoform production of *A. taxiformis*, using four LED lights (White, Red, Green, and Blue). Throughout both spectral light experiments, weekly growth rates were ~ 50 %/week (averaged 49.9 % and 51.5 % for the first and second experiment, respectively) and were not significantly affected by LED light variation across all weeks and treatments (Fig. 4c). However, different contamination levels developed under different LED lights over the course of experiments. In both LED experiments, red LED cultures were significantly more contaminated after 21 days in comparison to green, white and blue LEDs (Fig. 4, Table 1). The contamination captured by our analysis was mostly cyanobacterial.

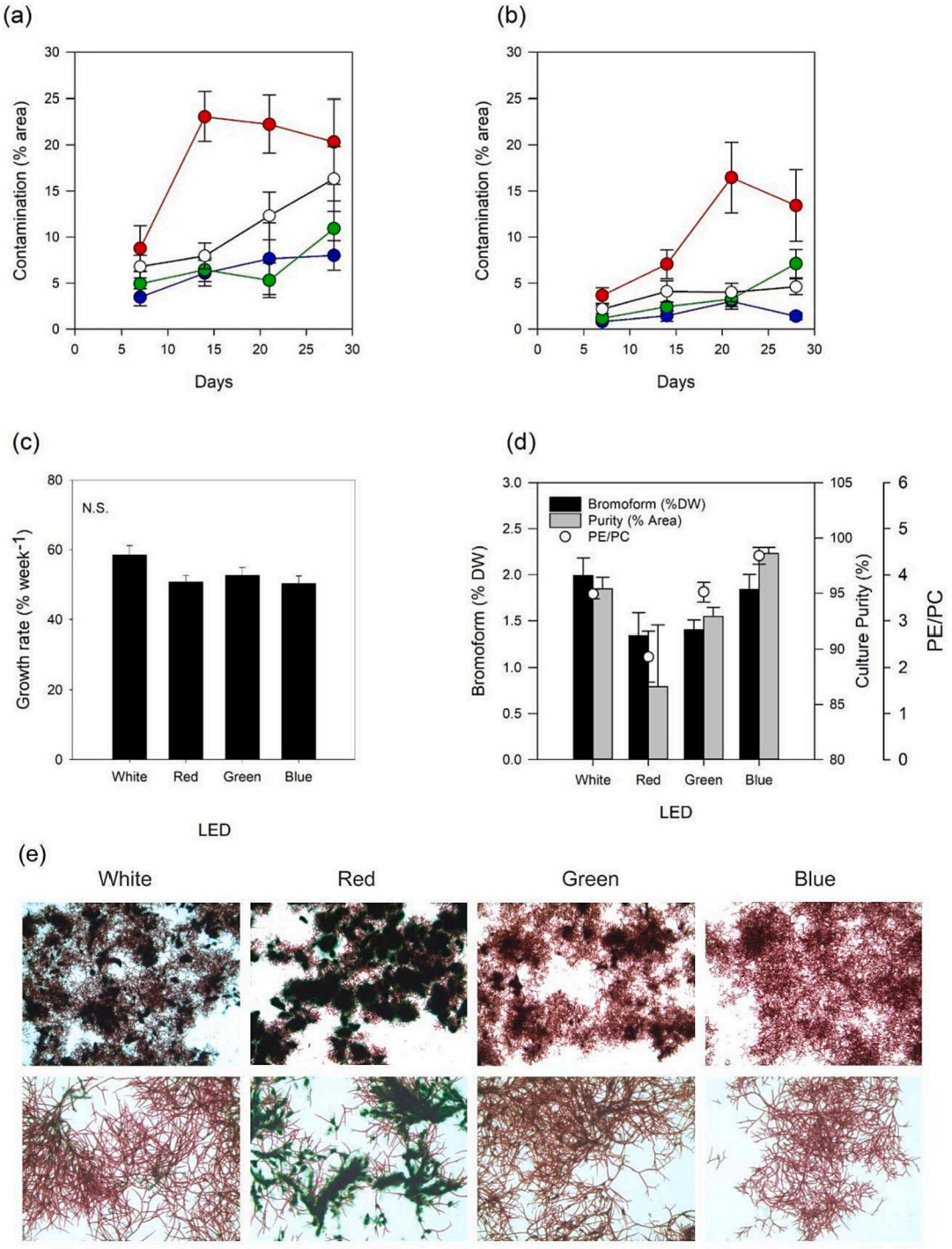

Fig. 4. Contamination (% area) levels in *A. taxiformis* cultures under different LED lights throughout two spectral light experiments. (a-b) Contamination throughout experiment #1 (a), and experiment #2 (b), each experiment started with different initial contaminating populations, $N = 6$, error bars represent standard error. (c) Growth rates for the four LED types tested in both experiments. Error bars represent standard error. No significant difference was found between growth in different LED lights. (d) Bromoform and culture purity (the opposite of contamination) together with the ratio of phycoerythrin (PE) to phycocyanin (PC), error bars represent standard error, $n = 6$. Bromofrom was only measured at the end of experiment #2 (e) Representative microscope pictures of *A. taxiformis* cultures illuminated by different LED lights at the end of spectral experiments #1 (top row) and #2 (bottom row). Pictures were taken at 10× and 25× magnification for experiment #1 and #2 respectively.

Red algae contamination (*Acrochaetium* sp.) was recorded in some cultures but was not quantified and did not seem to be affected by the change of LED lights.

Bromoform levels at the end of the experiments did not reveal significant differences between the spectral light treatments (ANOVA, $p = 0.051$). However, there was generally a positive relationship between culture purity (opposite of contamination) and bromoform concentration, with lower bromoform content for red LED cultures and higher bromoform content for blue LED cultures (Fig. 4d). Bromoform concentration in algal tissue ranged from a mean of 1.4 % DW (14 mg/g) in red LED cultures to over 2 % DW (20 mg/g) in white and blue LED cultures. Bulk pigment measurements showed a trend of increase in

phycocyanin in red LED cultures which led to a higher phycocyanin/phycoerythrin ratio (PC/PE) in cultures with higher contamination levels. In the red LED cultures, heavy contamination led to a characteristic change in spectral absorption profiles, with lower relative absorption in the phycoerythrin range and higher absorption in the phycocyanin range (Supplementary Online Material). Photosynthesis measurements showed low photosynthetic rates under red LED illumination in comparison to white, green, and blue LEDs (Supplementary Online Material).

3.3. Day length experiments

We explored the effect of day length (8, 12, 16, and 24 h light) on growth and reproduction of *A. taxiformis* and across all treatments, growth was highest at the default 12-h day/night treatment. Growth rates were significantly lower (Table 1) when day length was shortened to 8 h but there was no effect observed when daylight periods were extended to 16 or 24 h (Fig. 5a). Extended day length led to negative effects, similar to excessive light exposure, with decreased maximum photosynthetic quantum yield (Fv/fm) and elevated contamination levels (Fig. 5b and c). However, these negative effects were not statistically significant for 16 and 24 h day lengths. The growth rates recorded in this experiment were comparable to the growth rates recorded in the light intensity experiments (various light levels on 12:12 light: dark cycle) when plotted together versus Daily Light Integral (DLI) values (Fig. 5d). Throughout the experiment, reproduction was only anecdotally observed and was not consistent across treatments (in no >2 out of 48 wells per treatment).

4. Discussion

The goals of this study were to provide data that could contribute to the development of an efficient and scalable land-based aquaculture industry for *Asparagopsis taxiformis*. Specifically, we investigated how this novel seaweed responded to three major aspects of the primary energy source for photosynthetic production: light intensity, light spectrum, and day length. Overall we characterized the optimal light and biomass density range for cultivation, and developed a model for the

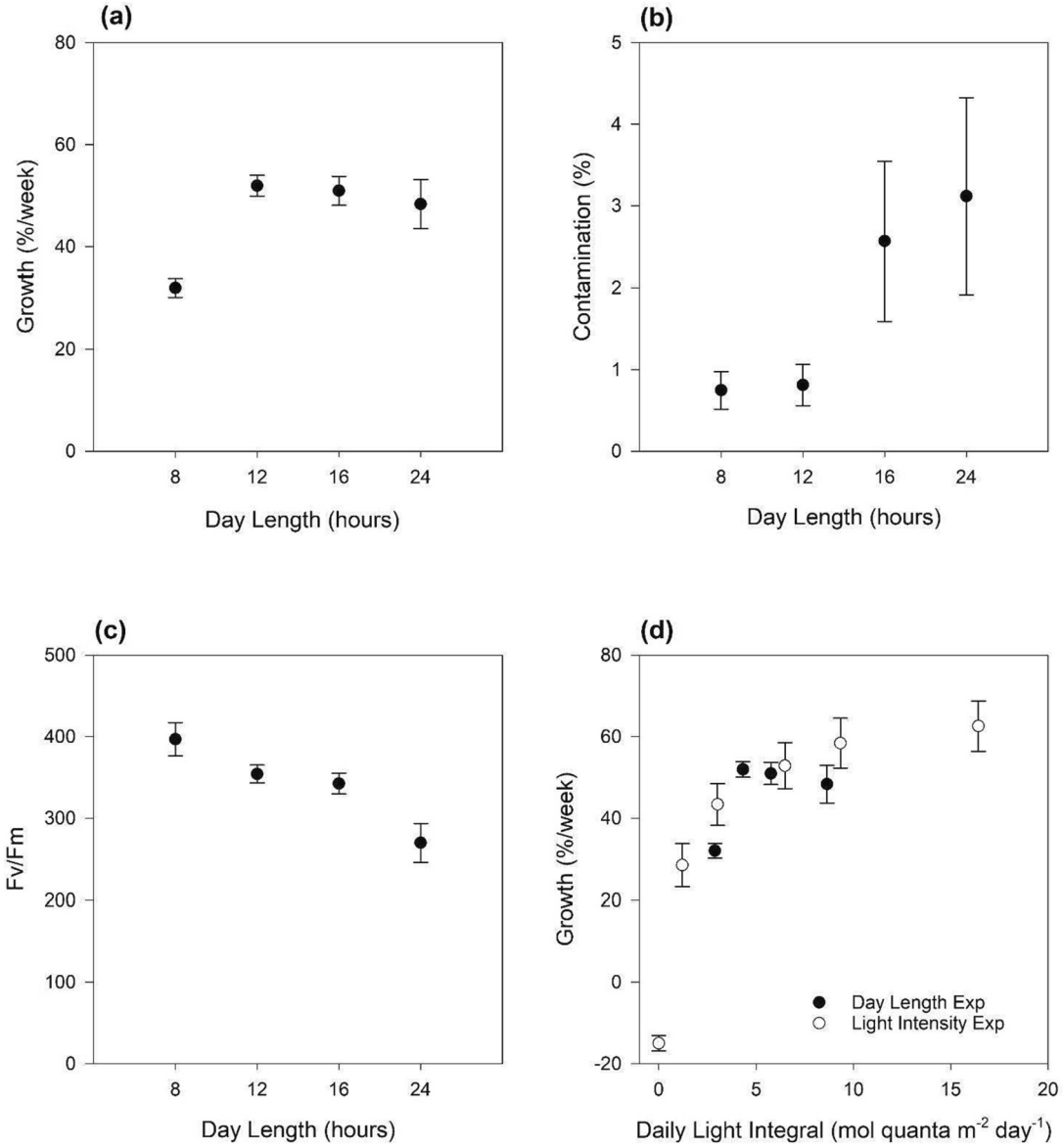

Fig. 5. Growth rates (a), contamination levels (b), and photosynthetic quantum yield (c) for *A. taxiformis* grown for 28 days in different day length treatments. Error bars represent standard error. $n = 6$. Panel (d) shows the growth data from the day length experiment (black circles) together with growth results from the light intensity experiments (open circles) versus the Daily Light Integral (DLI).

optimization of the light path in a cultivation vessel. Spectral light optimization revealed a significant reduction in cyanobacterial contamination for cultures illuminated with blue LED light. Artificially extending light duration longer than 12 h per day did not improve algal growth. In general, bromoform concentrations were highly variable but showed some trends in response to light treatments. Specifically, at low densities bromoform was highest in low light treatments while the opposite was true for higher density cultures. Further we saw highest production rates of bromoform in algae grown in either white or blue light suggesting that culture conditions can impact production or storage of key bioactive compounds.

4.1. Light intensity

Based on our light intensity investigations, we determined the compensation irradiance (I_c) and saturating irradiance (I_k) for *A. taxiformis* cultures inoculated at 1 gFW/l density, were ~ 6 μmol quanta m^{-2} s^{-1} and ~ 200 μmol quanta m^{-2} s^{-1}, respectively. PFD higher than 400 μmol quanta m^{-2} s^{-1} caused photoinhibition and often resulted in high contamination levels. This is in accordance with previous values reported for low density cultures of *Asparagopsis* [22,24] as well as with the conventional assumption of optimal light intensity of about 200–400 μmol quanta m^{-2} s^{-1} for most microalgae strains ((Masojídek et al. [25] and references within). I_k values increased with increasing biomass density reaching I_k ~ 700 μmol quanta m^{-2} s^{-1} for the highest density of 4 gFW/l coinciding with previous I_k values reported for *Asparagopsis* [23] based on variable chlorophyll fluorescence measurements.

In order to incorporate the effect of biomass density into predictions of *A. taxiformis* growth rates at given light intensities in the laboratory (vessels size up to 2 l), an empirical model was developed (*ATAXlab*). Building upon this model we infer that for PFD levels lower than ~200 μmol quanta m^{-2} s^{-1}, both growth rates and biomass production would not be improved by increasing the density higher than 1 gFW/l. However, for PFD > 400 μmol quanta m^{-2} s^{-1}, increased density is predicted to yield higher biomass production (although growth rates will not increase) coinciding with the high Optimal Culture Density (5 gFW/l) reported by [15] for outdoor *Asparagopsis* cultures under direct full sunlight. Hence, on a lab scale, with PFD lower than 400 μmol quanta m^{-2} s^{-1}, *A. taxiformis* should be cultivated at densities lower than 2 gFW/l to achieve the highest growth rates (%/week). However, biomass production rates (gFW/l/week) in these conditions are independent of density in the range of 1–4 gFW/l. This dictates that when biomass availability is the limiting factor (i.e. during the upscaling phase) low densities should be preferred, while when culture volume is limited (i.e. in full-capacity production) high densities may be used. Even though the use of high densities (>2 gFW/l) is not expected to result in improved biomass production, it may be beneficial for culture stability and lower contamination risk [15,39] and thus may be preferable for those reasons.

Extrapolating our findings to a more commercial application in large scale production systems requires the consideration of the variation in the light field experienced by *Asparagopsis* sporophytes as they are traveling through a cultivation tank. For that, light levels through a given path length were modeled based on Beer-Lambert law to simulate the light field in a cultivation system illuminated by a unidirectional light source (e.g. open pond illuminated from above or indoor tank illuminated from one side). Interestingly, we found that the biomass specific light attenuation coefficient (K_a) varied significantly between *A. taxiformis* cultures of different growth forms, with lower K_a for puffballs in comparison to filamentous morphologies (K_a ~ 0.025 and 0.035 m^2/gDW respectively). These values are in accordance with previous studies [22] where light attenuation values corresponding to K_a of ~0.03 m^2/gDW were reported for the "pompom" morphology of *Asparagopsis*. Incorporating this morphology-dependent K_a difference into our *ATAXout* model resulted in significant effects on biomass production predictions. While a low K_a puffball culture is predicted to yield the highest productivity of ~330 gFW m^{-2} $week^{-1}$ when grown at 1 gFW/l in a ~ 90 cm deep tank or at higher density of 3–5 gFW/l in a shallower ~20–40 cm tank, cultivating a high-K_a filamentous culture will yield only 230 gFW m^{-2} $week^{-1}$ and be limited to 30 cm depth if the density is 3 gFW/l or higher. For indoor cultivation systems illuminated with a PFD of 400 μmol quanta m^{-2} s^{-1}, the optimal light path length for a density > 3 gFW/l would be lower than 25 cm even when cultivating a low K_a culture.

These findings are generally in agreement with the prevalent microalgae PBR (photobioreactor) concept calling for the reduction of light-path as much as is practically feasible, when the biomass density and the mixing rate have been adjusted to their respective optimal values (e.g. [25,38,40]) This concept is exemplified in the extreme case of ultra-thin PBR designs, where the light path is reduced to <2 cm. For *Asparagopsis* however, the minimal feasible light path is significantly higher than for microalgae due to the alga's filamentous morphology. The OCD calculated here for a 20 cm deep *A. taxiformis* pond is ~3 gFW/l (~1 gDW/l) and ~ 4 gFW/l (~1.4 gDW/l) for high and low K_a cultures respectively. This is higher than the empirical OCD range reported for shallow microalgae ponds with comparable depth [25]). Schuenhoff et al. [15] reported OCD of 4 gFW/l (but with lower DW:FW ratio, translated to ~1gDW/l) for *A. armata* grown in 48 cm deep tanks (whereas the *ATAXout* model predicts OCD of ~0.7 gDW/l for a 48 cm light path with low K_a cultures). This apparent underestimation in our model might be the outcome of light penetration from the sides of the white polyethylene tanks used for cultivation by Schuenhoff et al. [15], practically reducing the average light path through the tank. Alternatively, the productivity of high-density cultures with a longer light path (tank depth) reported by Schuenhoff et al. [15] may be the outcome of an even lower biomass specific light attenuation (K_a) allowing light to penetrate deeper into the culture.

Overall, the K_a value found here for *A.taxiformis* puffball/pompom cultures is lower than K_a values reported for microalgae (e.g. [41–43] and may represent an important advantage for large scale cultivation. One approach to improve the efficiency of light utilization in microalgae cultures is to minimize the size of light-harvesting antennae through genetic modification. This is thought to reduce the absorbance of light by the outer layers of algae culture, which can help to prevent the dissipation of light through non-photochemical quenching (NPQ) and reduce the risk of photoinhibition. Minimizing the size of the antennae may also allow more light to reach deeper layers of the algae, which can improve the overall efficiency of light utilization for photosynthesis [44,45]. For *A. taxiformis* sporophytes, the compacted puffball morphology may work in a similar way, and might be manipulated by cultivation conditions rather than by genetic modification. A potentially important aspect of the light environment in a cultivation setting is drastic changes in light intensity as the algae are mixed via bubbling. Light fluctuation, with frequency on the scale of tens of Hz was shown in some instances to increase productivity by >50 % [46]. However, for cultivation systems with light paths longer than a few cm, fluctuating light effect is expected to lose its significance [38] and is therefore considered of lower relevance to the model presented here. The effect of light and density on bromoform content is still not clear and is probably based on more than one mechanism (possibly higher bromoform release in high light (as noted by [31,32]) and lower bromoform production due to Br^- limitation in higher stocking densities) and needs to be further investigated.

4.2. Light spectrum

Asparagopsis, as with all red algae are able to absorb light over most of the PAR spectrum, including spectral ranges that are not available for most other photosynthetic organisms [27]. Our findings suggest that *Asparagopsis* cultures may efficiently utilize blue light (at the longer end of the blue band, λ = 480 ± 20 nm) to outcompete potential

cyanobacterial competitors. More specifically, avoiding the use of red light (600-700 nm) in culture illumination was found to be effective at controlling cyanobacterial growth (which may negatively influence culture performance through competition for resources such as light and nutrients). This finding was supported by (1) a 35 day experiment started with only cryptic cyanobacterial contamination, (2) 28 days experiment started with known cyanobacterial contamination, and (3) spectral absorption and oxygen evolution measurements. It is important to note that many cyanobacteria may also possess the red pigment phycoerythrin [47] and may undergo chromatic adaptation in response to changes in spectral illumination, shifting from red to green light absorption by producing more phycoerythrin (PE) than phycocyanin (PC) [48]. In our experiments, the filamentous cyanobacteria residing in *A. taxiformis* cultures were observed to change their color from pale green to bright turquoise when illuminated with blue and red light respectively, indicating higher phycocyanin content. However, even with this capability to change their pigment profile, these cyanobacteria only outcompeted *A. taxiformis* when illuminated with red LED or the cold white LED (which also included a red emission band). A high ratio of PC to PE was found to be related to contamination, suggesting this pigment ratio might be used as an indicator of cyanobacterial contamination in *A. taxiformis* cultures. However, the known capability of both cyanobacteria and red algae to change their pigment profiles in response to a change in light and nutrient availability [48,49] should also be taken into account while attempting this approach.

Bromoform content was found to be lowest for cultures illuminated by red LED and was inversely correlated with contamination levels representing a dilution of bromoform-rich *A. taxiformis* by bromoform-depleted contaminants. Our findings suggest that spectral light range may be used to control cyanobacteria contamination by using a blue light source (~450–500 nm) for culture illumination indoors or by wrapping outdoor cultures with spectral filters to screen orange-red light at wavelengths >600 nm. Blue shading nets might be applicable for this purpose as well, but probably with less success since some orange-red light is usually transmitted [50].

4.3. Day length

From an energy source perspective, light duration (i.e. day length) is an extremely important parameter affecting the integrated PFD available for photosynthesis over course of a day (Daily Light Integral; DLI). Nevertheless, from a biological point of view, extended day length may have a more complex effect on photosynthetic organisms, including their reproduction and/or metabolism [29]. We found a significant increase in the growth rate of *A. taxiformis* when the day length was extended from 8 to 12 h, but no further improvements in growth for further increases to 16 and 24 h. The improvement in growth when comparing 8 to 12 h day length coincides with the increase in DLI based on the curves measured for 1 gFW/l cultures in this study. However, increased DLI for longer day length (16 and 24 h) did not yield the expected growth improvements and resulted in compromised photosynthetic efficiency and higher contamination susceptibility, both indicating some levels of photoinhibition. Reproduction was not induced when day length was shortened, probably due to the warm temperature (23 °C).

Based on these findings, the extension of day length beyond 12 h is not an efficient strategy for improving *A. taxiformis* production. It should be noted however, that it might be effective for *A. taxiformis* grown at very low light or high density where light availability poses a severe energetic limitation.

5. Conclusions

To effectively address the necessity for methane emission reduction in ruminants, there's a pressing need to expedite the optimization of the emerging *Asparagopsis* cultivation industry. This optimization should primarily focus on (1) enhancing the rate of biomass production and (2) elevating the bromoform content within this biomass. This is pivotal because the dosage of *Asparagopsis* in the diet of ruminants is determined by the bromoform levels present, and an increase in bromoform content would translate into a reduced requirement for biomass to achieve methane emission reduction objectives. For optimal biomass production, *A. taxiformis* may be efficiently cultivated in the lab at light levels ~200 μmol quanta m^{-2} s^{-1}, at density of ~1 gFW/l, while at outdoor light levels of 2000 μmol quanta m^{-2} s^{-1}, the highest productivity may be achieved for a wide range of biomass densities up to 7 gFW/l by designing a system with the right light path (from ~20 cm for 7 gFW/l to ~70 cm for 1 gFW/l). The biomass specific light attenuation (K_a) has a major impact on production capacity and is lower for cultures of the puffball/pompom morphology. Improved light penetration may be achieved by using low K_a (0.025 m^2/g) cultures (e.g. cultures of the "pompom" morphology), a strategy which is predicted to increase production by ~40 % in comparison to higher "normal" K_a (0.035 m^2/g) cultures. While maximizing biomass production may be achieved through increasing incident PAR levels, it should be noted that excessive light may lead to elevated contamination risk and bromoform loss. The use of blue LED for illumination or screening out light of wavelengths longer than 600 nm represents an efficient strategy to at least partially control cyanobacteria in *A. taxiformis* cultures and may be used as part of future pest management plans. Artificially extending day length beyond 12 h is not recommended for *A. taxiformis* cultivation and may result in high-light stress symptoms when extended beyond 16 h. The results presented here offer suggestions for the design of land-based *A. taxiformis* cultivation systems that optimize the light environment for biomass production and bromoform content. These results will help to inform the development of commercial scale production of *A. taxiformis* to ensure that it is accessible as a methane mitigating resource to the livestock industry.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gal Dishon reports financial support was provided by Blue Ocean Barns. Gal Dishon reports a relationship with Blue Ocean Barns that includes: employment.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank Samantha Clements for assistance with all lab-related work. The authors thank Blue Ocean Barns Inc., the Builders Initiative, and the National Science Foundation (award 2129490 to V.A. and J.E.S).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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